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PHARMACIA & UPJOHN COMPANY [/]; (). GURNEY, Mark, E. [/]; (). BIENKOWSKI, Michael, Jerome [/]; (). HEINRIKSON, Robert, Leroy [/]; (). PARODI, Luis, A. [/]; (). YAN, Riqiang [/]; (). GURNEY, Mark, E. [/]; (). BIENKOWSKI, Michael, Jerome [/]; (). HEINRIKSON, Robert, Leroy [/]; (). PARODI, Luis, A. [/]; (). YAN, Riqiang [/]; (). WOOTTON, Thomas, A. ; ().

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(54) Title: ALZHEIMER'S DISEASE SECRETASE

(54) Titre: SECRETASE DE LA MALADIE D'ALZHEIMER

(57) Abstract

The present invention provides the enzyme and enzymatic procedures for cleaving the 'beta' secretase cleavage site of the APP protein and associated nucleic acids, peptides, vectors, cells and cell isolates and assays.

(57) Abrégé

La présente invention porte sur l'enzyme et les procédures enzymatiques de clivage du site de clivage de la 'beta' secrétase de la protéine APP et des acides nucléiques, des peptides, des vecteurs, des cellules et des isolats cellulaires associés, et sur des dosages.

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(71) Applicant *(for all designated States except US)*: PHARMACIA & UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: GURNEY, Mark, E. [US/US]; 910 Rosewood Avenue, S.E., Grand Rapids, MI 49506 (US). BIENKOWSKI, Michael, Jerome [US/US]; 3431 Hollow Wood, Portage, MI 49024 (US). HEINRIKSON, Robert, Leroy [US/US]; 81 South Lake Doster Drive, Plainwell, MI 49080 (US). PARODI, Luis, A. [US/SE]; Grevgafan 24, S-115 43 Stockholm (SE). YAN, Riqiang [US/US]; 5026 Queen Victoria Street, Kalamazoo, MI 49009 (US).

(74) Agent: WOOTTON, Thomas, A.; Pharmacia & Upjohn Company, Intellectual Property Legal Services, 301 Henrietta Street, Kalamazoo, MI 49001 (US).

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(54) Title: ALZHEIMER'S DISEASE SECRETASE

(57) Abstract

The present invention provides the enzyme and enzymatic procedures for cleaving the β secretase cleavage site of the APP protein and associated nucleic acids, peptides, vectors, cells and cell isolates and assays.

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(74) Agent: **WOOTTON, Thomas, A.**; Pharmacia & Upjohn Company, Intellectual Property Legal Services, 301 Henrietta Street, Kalamazoo, MI 49001 (US).

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(57) Abstract: The present invention provides the enzyme and enzymatic procedures for cleaving the β secretase cleavage site of the APP protein and associated nucleic acids, peptides, vectors, cells and cell isolates and assays.

AMENDED CLAIMS

[received by the International Bureau on 2 October 2000 (02.10.00);
original claims 1-141 replaced by new claims 1-150 (18 pages)]

1. A purified polypeptide comprising a mammalian Asp2 polypeptide that cleaves a mammalian β-amyloid precursor protein (APP), or a fragment, analog, or derivative of said mammalian Asp2 polypeptide that retains the APP cleaving activity.

2. A purified polypeptide according to claim 1, selected from the group consisting of:

(a) a polypeptide comprising a purified human Asp2(a) amino acid sequence set forth in SEQ ID NO: 4 or a fragment thereof that cleaves APP;

(b) a polypeptide comprising a purified human Asp2(b) amino acid sequence set forth in SEQ ID NO: 6 or a fragment thereof that cleaves APP;

(c) a polypeptide comprising the murine Asp2 amino acid sequence set forth in SEQ ID NO: 8, or a fragment thereof that cleaves APP;

(d) a polypeptide comprising a purified polypeptide having an amino acid sequence that is at least 95% identical to (a), (b), or (c).

3. A purified polypeptide according to claim 1, comprising a purified human Asp2(a) amino acid sequence set forth in SEQ ID NO: 4 or a fragment thereof that cleaves APP.

4. A purified polypeptide according to claim 1, said polypeptide comprising a portion of the human Asp2(a) amino acid sequence set forth in SEQ ID NO: 4, said portion including amino acids 22-501 of SEQ ID NO: 4 and lacking amino acids 1-21.

5. A purified polypeptide according to claim 1, said polypeptide comprising a portion of the human Asp2(a) amino acid sequence set forth in SEQ ID NO: 4 effective to cleave APP, said polypeptide lacking transmembrane domain amino acid residues 455-477 of SEQ ID NO: 4.

6. A polypeptide according to claim 5, said polypeptide lacking amino acids 454-501 of SEQ ID NO: 4.

7. A purified polypeptide according to claim 1, comprising a purified human Asp2(b) amino acid sequence set forth in SEQ ID NO: 6 or a fragment thereof that cleaves APP.

8. A purified polypeptide according to claim 1, said polypeptide comprising a portion of the human Asp2(b) amino acid sequence set forth in SEQ ID NO: 6, said portion including amino acids 22-476 of SEQ ID NO: 6 and lacking amino acids 1-21.

9. A purified polypeptide according to claim 1, said polypeptide comprising a portion of the human Asp2(b) amino acid sequence set forth in SEQ ID NO: 6 effective to cleave APP, said polypeptide lacking transmembrane domain amino acid residues 430-452 of SEQ ID NO: 6.

10. A purified polypeptide according to claim 1, comprising the murine Asp2 amino acid sequence set forth in SEQ ID NO: 8, or a fragment thereof that cleaves APP.

11. A purified polypeptide according to claim 1 comprising a fragment of a mammalian Asp2 polypeptide, wherein the purified polypeptide lacks the transmembrane domain of said mammalian Asp2 polypeptide.

12. A fusion protein comprising a polypeptide according to any one of claims 1-10, and which further includes a heterologous tag amino acid sequence.

13. A polypeptide according to any one of claims 1-12, wherein the polypeptide cleaves human APP or human APP-Sw at the β-secretase recognition site.

14. A polypeptide according to any one of claims 1-3, 5-7, or 9-13, wherein the polypeptide lacks any mammalian Asp2 pro-peptide sequence.

15. A polypeptide according to claim 14, beginning with the N-terminal sequence ETDEEP.

16. A polypeptide according to any one of claims 1-3, 5-7, 9, or 11-15, selected from the group consisting of:

(a) a polypeptide comprising a portion of the amino acid sequence set forth in SEQ ID NO: 4 effective to cleave APP, wherein the polypeptide lacks amino acids 1-45 of SEQ ID NO: 4; and

(b) a polypeptide comprising a portion of the amino acid sequence set forth in SEQ ID NO: 6 effective to cleave APP, wherein the polypeptide lacks amino acids 1-45 of SEQ ID NO: 6.

17. A purified polynucleotide comprising a nucleotide sequence that encodes a polypeptide according to any one of claims 1 to 16.

18. A polynucleotide according to claim 17, selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO: 3;

(b) a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO: 5;

(c) a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO: 7;

(d) a polynucleotide comprising a nucleotide sequence that is at least 95% identical to (a), (b), or (c), and that encodes a polypeptide that cleaves APP; and

(e) a fragment of (a), (b), (c), or (d) that encodes a polypeptide that cleaves APP.

19. A polynucleotide according to claim 17 comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 21, 23, 25, 27, 29, and 31.

20. A purified polynucleotide according to claim 17, selected from the group consisting of:

(a) a purified polynucleotide that comprises a nucleotide sequence that encodes amino acids 22-501 of SEQ ID NO: 4 and lacks adjacent nucleotide sequence encoding amino acids 1-21 of SEQ ID NO: 4; and

(b) a purified polynucleotide that comprises a nucleotide sequence that encodes amino acids 22-476 of SEQ ID NO: 6 and lacks adjacent nucleotide sequence encoding amino acids 1-21 of SEQ ID NO: 6.

21. A purified polynucleotide according to claim 17, selected from the group consisting of:

(a) a purified polynucleotide comprising a nucleotide sequence that encodes a portion of the human Asp2(a) amino acid sequence set forth in SEQ ID NO: 4 effective to cleave APP, and wherein the polynucleotide lacks adjacent nucleotide sequence encoding transmembrane domain amino acid residues 455-477 of SEQ ID NO: 4; and

(b) a purified polynucleotide comprising a nucleotide sequence that encodes a portion of the human Asp2(a) amino acid sequence set forth in SEQ ID NO: 6 effective to cleave APP, and wherein the polynucleotide lacks adjacent nucleotide sequence encoding transmembrane domain amino acid residues 430-452 of SEQ ID NO: 6.

22. A purified polynucleotide according to claim 21, said polynucleotide lacking nucleotide sequence encoding amino acids 454-501 of SEQ ID NO: 4.

23. A purified polynucleotide according to claim 17 comprising a fragment of a mammalian Asp2 polynucleotide, wherein the fragment lacks nucleotide sequence encoding the transmembrane domain of said mammalian Asp2 polypeptide.

24. A purified polynucleotide according to claim 17, wherein the polynucleotide lacks a nucleotide sequence encoding a mammalian Asp2 pro-peptide sequence.

25. A vector comprising a polynucleotide according to any one of claims 17-24.

26. A vector according to claim 25 that is an expression vector wherein the polynucleotide is operably linked to an expression control sequence.

27. A host cell transformed or transfected with a polynucleotide according to any one of claims 17-24.

28. A host cell transformed or transfected with a vector according to claim 25 or 26.

29. A host cell according to claim 28 that is a mammalian cell.

30. A host cell according to claim 28 or 29 that expresses the polypeptide on its surface.

31. A host cell according to claim 28 or 29 that secretes the polypeptide encoded by the polynucleotide, wherein the secreted polypeptide lacks a transmembrane domain.

32. A host cell according to any one of claims 27-31, wherein the host cell is transfected with a nucleic acid comprising a nucleotide sequence that encodes an amyloid precursor protein (APP) or fragment thereof that includes a protease recognition site recognized by the polypeptide.

33. A host cell according to claim 32, wherein the host cell is transfected with a nucleic acid comprising a nucleotide sequence that encodes an amyloid precursor protein (APP).

34. A host cell according to claim 33, wherein the host cell is transfected with a nucleic acid comprising a nucleotide sequence that encodes an amyloid precursor protein (APP) that includes two carboxy-terminal lysine residues.

35. A host cell according to any one of claims 32-34, wherein the APP or fragment thereof includes the APP Swedish mutation sequence KM→NL immediately upstream of the β-secretase cleavage site.

36. A host cell according to any one of claims 32-35 that expresses the polypeptide and the APP or APP fragment on its surface.

37. A method of making a polypeptide that cleaves APP, comprising steps of culturing a host cell according to any one of claims 27-36 in a culture medium under conditions in which the cell produces the polypeptide that is encoded by the polynucleotide.

38. A method according to claim 37, further comprising a step of purifying the polypeptide from the cell or the culture medium.

39. A method for identifying agents that inhibit the activity of human Asp2 aspartyl protease (Hu-Asp2), comprising the steps of:

(a) contacting amyloid precursor protein (APP) and a polypeptide according to any one of claims 1-16 in the presence and absence of a test agent;

(b) determining the APP processing activity of the polypeptide in the presence and absence of the test agent; and

(c) comparing the APP processing activity of the polypeptide in the presence of the test agent to the activity in the absence of the test agent to identify an agent that inhibits the APP processing activity of the polypeptide, wherein reduced activity in the presence of the test agent identifies an agent that inhibits Hu-Asp2 activity.

40. A method according to claim 39, wherein the polypeptide is a recombinant polypeptide purified and isolated from a cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes the polypeptide.

41. A method according to claim 39,

wherein the polypeptide is expressed in a cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes the polypeptide,

wherein the contacting comprises growing the cell in the presence and absence of the test agent, and

wherein the determining step comprises measuring APP processing activity of the cell.

42. A method according to claim 41, wherein the determining step comprises measuring the production of amyloid beta peptide by the cell in the presence and absence of the test agent.

43. A method according to claim 41 or 42, wherein the cell is a human embryonic kidney cell line 293 (HEK293) cell.

44. A method according to any one of claims 40-43 wherein the nucleotide sequence is selected from the group consisting of:

(a) a nucleotide sequence encoding the Hu-Asp2(a) amino acid sequence set forth in SEQ ID NO: 4;

(b) a nucleotide sequence encoding the Hu-Asp2(b) amino acid sequence set forth in SEQ ID NO: 6;

(c) a nucleotide sequence encoding a fragment of Hu-Asp2(a) (SEQ ID NO: 4) or Hu-Asp2(b) (SEQ ID NO: 6), wherein said fragment exhibits aspartyl protease activity characteristic of Hu-Asp2(a) or Hu-Asp2(b); and

(d) a nucleotide sequence of a polynucleotide that hybridizes under stringent hybridization conditions to a Hu-Asp2-encoding polynucleotide selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 5.

45. A method according to any one of claims 40-43, wherein the Hu-Asp2 comprises the Hu-Asp2(a) amino acid sequence set forth in SEQ ID NO: 4.

46. A method according to any one of claims 40-43, wherein the Hu-Asp2 comprises the Hu-Asp2(b) amino acid sequence set forth in SEQ ID NO: 6.

47. A method according to any one of claims 40-43, wherein the Hu-Asp2 comprises a fragment of Hu-Asp2(a) (SEQ ID NO: 4) or Hu-Asp2(b) (SEQ ID NO: 6), wherein said fragment exhibits aspartyl protease activity characteristic of Hu-Asp2(a) or Hu-Asp2(b).

48. A method according to any one of claims 40-47, wherein the cell comprises a vector that comprises the polynucleotide.

49. A method according to any one of claims 39-48, wherein the APP comprises the Swedish mutation (K→ N, M→ L) adjacent to the β-secretase processing site.

50. A method according to any one of claims 39-49, wherein the APP further comprises a carboxy-terminal di-lysine.

51. A method for identifying agents that modulate the activity of Asp2 aspartyl protease, comprising the steps of:

(a) contacting a purified and isolated polypeptide according to any one of claims 1-16 and amyloid precursor protein (APP) in the presence and absence of a test agent, wherein the Asp2 aspartyl protease is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a Hu-Asp2-encoding polynucleotide selected from the group consisting of SEQ ID NO: 4 and SEQ ID NO: 6;

(b) determining the APP processing activity of the polypeptide in the presence and absence of the test agent; and

(c) comparing the APP processing activity of the polypeptide in the presence of the test agent to the activity in the absence of the agent to identify agents that modulate the activity of the polypeptide, wherein a modulator that is an Asp2 inhibitor reduces APP processing and a modulator that is an Asp2 agonist increases such processing.

52. A method according to any one of claims 39-51, further comprising a step of treating Alzheimer's Disease with an agent identified as an inhibitor of Hu-Asp2 according to steps (a)-(c).

53. The use of an agent identified as an inhibitor of Hu-Asp2 according to any one of claims 39-41 in the manufacture of a medicament for the treatment of Alzheimer's Disease.

54. A method for assaying for modulators of β-secretase activity, comprising the steps of:

(a) contacting a first composition with a second composition both in the presence and in the absence of a putative modulator compound, wherein the first composition comprises a polypeptide according to any one of claims 1-16, and wherein the second composition comprises a substrate polypeptide having an amino acid sequence comprising a β-secretase cleavage site;

(b) measuring cleavage of the substrate polypeptide in the presence and in the absence of the putative modulator compound; and

(c) identifying modulators of β-secretase activity from a difference in cleavage in the presence versus in the absence of the putative modulator compound, wherein a modulator that is a β-secretase antagonist reduces such cleavage and a modulator that is a β-secretase agonist increases such cleavage.

55. A method according to claim 54, wherein the polypeptide of the first composition comprises a polypeptide purified and isolated from a cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes the polypeptide.

56. A method according to claim 54, wherein the polypeptide of the first composition is expressed in a cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes the polypeptide, and wherein the measuring step comprises measuring APP processing activity of the cell.

57. A method according to claim 54, wherein the first composition comprises a purified human Asp2 polypeptide.

58. A method according to claim 54, wherein the first composition comprises a soluble fragment of a human Asp2 polypeptide that retains Asp2 β-secretase activity.

59. A method according to claim 58 wherein the soluble fragment is a fragment lacking an Asp2 transmembrane domain.

60. A method according to claim 58, wherein the substrate polypeptide of the second composition comprises the amino acid sequence SEVNLDAEFR.

61. A method according to claim 58, wherein the substrate polypeptide of the second composition comprises the amino acid sequence EVKMDAEF.

62. A method according to claim 58, wherein the second composition comprises a polypeptide having an amino acid sequence of a human amyloid precursor protein (APP).

63. A method according to claim 62, wherein the human amyloid precursor protein is selected from the group consisting of: APP695, APP751, and APP770.

64. A method according to claim 63, wherein the human amyloid precursor protein includes at least on mutation selected from a KM→ NL Swiss mutation and a V→ F London mutation.

65. A method according to claim 62, wherein the polypeptide having an amino acid sequence of a human APP further comprises an amino acid sequence comprising a marker sequence attached amino-terminal to the amino acid sequence of the human amyloid precursor protein.

66. A method according to claim 62, wherein the polypeptide having an amino acid sequence of a human APP further comprises two lysine residues attached to the carboxyl terminus of the amino acid sequence of the human APP.

67. A method according to claim 54, wherein the second composition comprises a eukaryotic cell that expresses amyloid precursor protein (APP) or a fragment thereof containing a β-secretase cleavage site.

68. A method according to claim 67, wherein the APP expressed by the host cell is an APP variant that includes two carboxyl-terminal lysine residues.

69. A method according to any one of claims 54-68, further comprising a step of treating Alzheimer's Disease with an agent identified as an inhibitor of Hu-Asp2 according to steps (a)-(c).

70. The use of an agent identified as an inhibitor of Hu-Asp2 according to any one of claims 54-68 in the manufacture of a medicament for the treatment of Alzheimer's Disease.

71. A method for identifying agents that inhibit the activity of human Asp2 aspartyl protease (Hu-Asp2), comprising the steps of:

(a) growing a cell in the presence and absence of a test agent, wherein the cell expresses a polypeptide according to any one of claims 1-16 and expresses an amyloid precursor protein (APP) that comprises a carboxy-terminal di-lysine (KK);

(b) determining the APP processing activity of the cell in the presence and absence of the test agent; and

(c) comparing the APP processing activity in the presence of the test agent to the activity in the absence of the test agent to identify an agent that inhibits the activity of Hu-Asp2, wherein reduced activity in the presence of the test agent identifies an agent that inhibits Hu-Asp2 activity.

72. A method according to claim 71, wherein the APP further comprises the Swedish mutation (K→ N, M→ L) adjacent to the β-secretase processing site.

73. A method according to claim 71 or 72, wherein the host cell has been transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes a Hu-Asp2, wherein said nucleotide sequence is selected from the group consisting of:

(a) a nucleotide sequence encoding the Hu-Asp2(a) amino acid sequence set forth in SEQ ID NO: 4;

(b) a nucleotide sequence encoding the Hu-Asp2(b) amino acid sequence set forth in SEQ ID NO: 6;

(c) a nucleotide sequence encoding a fragment of Hu-Asp2(a) (SEQ ID NO: 4) or Hu-Asp2(b) (SEQ ID NO: 6), wherein said fragment exhibits aspartyl protease activity characteristic of Hu-Asp2(a) or Hu-Asp2(b); and

(d) a nucleotide sequence of a polynucleotide that hybridizes under stringent hybridization conditions to a Hu-Asp2-encoding polynucleotide selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 5.

74. A method according to any one of claims 71-73, further comprising a step of treating Alzheimer's Disease with an agent identified as an inhibitor of Hu-Asp2 according to steps (a)-(c).

75. The use of an agent identified as an inhibitor of Hu-Asp2 according to any one of claims 71-73 in the manufacture of a medicament for the treatment of Alzheimer's Disease.

76. A method of reducing cellular production of amyloid beta (Aβ) from amyloid precursor protein (APP), comprising step of transforming or transfecting cells with an anti-sense reagent capable of reducing Asp2 polypeptide production by reducing Asp2 transcription or translation in the cells, wherein reduced Asp2 polypeptide production in the cells correlates with reduced cellular processing of APP into Aβ .

77. A method of reducing cellular production of amyloid beta (Aβ) from amyloid precursor protein (APP), comprising steps of:

(a) identifying mammalian cells that produce Aβ ; and

(b) transforming or transfecting the cells with an anti-sense reagent capable of reducing Asp2 polypeptide production by reducing Asp2 transcription or translation in the cells, wherein reduced Asp2 polypeptide production in the cells correlates with reduced cellular processing of APP into Aβ .

78. A method according to claim 77, wherein the identifying step comprises diagnosing Alzheimer's disease, where Alzheimer's disease correlates with the existence of cells that produce Aβ that forms amyloid plaques in the brain.

79. A method according to any one of claims 76-78, wherein the cell is a neural cell.

80. A method according to any one of claims 76-79, wherein the anti-sense reagent comprises an oligonucleotide comprising a single stranded nucleic acid sequence capable of binding to a Hu-Asp mRNA.

81. A method according to any one of claims 76-80, wherein the anti-sense reagent comprises an oligonucleotide comprising a single stranded nucleic acid sequence capable of binding to a Hu-Asp DNA.

82. A polypeptide comprising the amino acid sequence of a mammalian amyloid protein precursor (APP) or fragment thereof containing an APP cleavage site recognizable by a mammalian β-secretase, and further comprising two lysine residues at the carboxyl terminus of the amino acid sequence of the mammalian APP or APP fragment.

83. A polypeptide according to claim 82 comprising the amino acid sequence of a mammalian amyloid protein precursor (APP), and further comprising two lysine residues at the carboxyl terminus of the amino acid sequence of the mammalian amyloid protein precursor.

84. A polypeptide according to claim 82 or 83, wherein the mammalian APP is a human APP.

85. A polypeptide according to any one of claims 82-84, wherein the human APP comprises at least one variation selected from the group consisting of a Swedish KM→NL mutation and a London V717→F mutation.

86. A polynucleotide comprising a nucleotide sequence that encodes a polypeptide according to any one of claims 82-85.

87. A vector comprising a polynucleotide according to claim 86.

88. A vector according to claim 87 wherein said polynucleotide is operably linked to a promoter to promote expression of the polypeptide encoded by the polynucleotide in a host cell.

89. A host cell transformed or transfected with a polynucleotide according to claim 86 or a vector according to claim 87 or 88.

90. A host cell according to claim 89 that is a mammalian cell.

91. An isolated nucleic acid molecule comprising a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), wherein said Hu-Asp1, Hu-Asp2(a) and Hu-Asp2(b) polypeptides have the complete amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID No:6, respectively; and

(b) a nucleotide sequence complementary to the nucleotide sequence of (a).

92. The nucleic acid molecule of claim 91, wherein said Hu-Asp polypeptide is Hu-Asp1.

93. The nucleic acid molecule of claim 91, wherein said Hu-Asp polypeptide is Hu-Asp2(a).

94. The nucleic acid molecule of claim 91, wherein said Hu-Asp polypeptide is Hu-Asp2(b).

95. An isolated nucleic acid molecule comprising polynucleotide which hybridizes under stringent conditions to a polynucleotide comprising a nucleotide sequence selected from:

(a) a nucleotide sequence encoding a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), wherein said Hu-Asp1, Hu-Asp2(a) and Hu-Asp2(b) polypeptides have the complete amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID No:6, respectively; and

(b) a nucleotide sequence complementary to the nucleotide sequence of (a).

96. A vector comprising the nucleic acid molecule of any one of claims 91-95.

97. The vector of claim 96, wherein said nucleic acid molecule is operably linked to a promoter for the expression of a Hu-Asp polypeptide.

98. A host cell comprising the vector of claim 96 or 97.

99. A method of obtaining a Hu-Asp polypeptide comprising culturing the host cell of claim 98 and isolating said Hu-Asp polypeptide.

100. An isolated Hu-Asp1 polypeptide comprising an amino acid sequence at least 95% identical to a sequence comprising the amino acid sequence of SEQ ID NO:2.

101. An isolated Hu-Asp2(a) polypeptide comprising an amino acid sequence at least 95% identical to a sequence comprising the amino acid sequence of SEQ ID NO:4.

102. An isolated Hu-Asp2(a) polypeptide comprising an amino acid sequence at least 95% identical to a sequence comprising the amino acid sequence of SEQ ID NO:8.

103. An isolated antibody that binds specifically to the Hu-Asp polypeptide of any of claims 100-102.

104. A cell according to claim 98 that is a bacterial cell.

105. A bacterial cell of claim 104 where the bacteria is *E coli*.

106. A cell according to any one of claims 27-36 or 98 that is a eukaryotic cell.

107. A cell according to any one of claims 27-36 or 98 that is an insect cell.

108. An insect cell of claim 107 where the insect is sf9, or High 5.

109. An insect cell of claim 107 where the insect cell is High 5.

110. A cell according to any one of claims 27-36 or 98 that is a mammalian cell.

111. A mammalian cell of claim 110 selected from the group consisting of human, rodent, lagomorph, and primate cells.

112. A mammalian cell of claim 111 that is a human cell.

113. A mammalian cell of claim 112 selected from the group consisting of HEK293 and IMR-32 cells.

114 A mammalian cell of claim 111 that is a primate cell.

115. A primate cell of claim 114 that is a COS-7 cell.

116. A mammalian cell of claim 111 that is a rodent cell.

117. A rodent cell of claim 116 selected from, CHO-K1, Neuro-2A, 3T3 cells.

118. A cell according to any one of claims 27-36 or 98 that is a yeast cell.

119. A cell according to any one of claims 27-36 or 98 that is an avian cell.

120. Any isoform of Amyloid Precursor Protein (APP) modified such that the last two carboxy terminus amino acids of that isoform are both lysine residues.

121. The isoform of APP from claim 130 comprising the isoform known as APP695 modified so that its last two carboxy terminus amino acids are lysines.

122. The isoform of claim 121 comprising SEQ. ID. 16.

123. The isoform variant of claim 121 comprising SEQ. ID. NO. 18 or 20.

124. A nucleic acid encoding a polypeptide according to any of claims 120-123.

125. An eukaryotic cell comprising a nucleic acids of claim 124.

126. An eukaryotic cell comprising a polypeptide of claim 120-123.

127. An eukaryotic cell according to claim 125 or 126 that is a mammaliam cell.

128. A mammalian cell according to claim 127, selected from the group consisting of HEK293 and Neuro2a.

129. A method according to any of claims 39, 41-50, 54, 56, and 71-73 in which the determining or measuring step comprises measuring the amount of amyloid beta-peptide released into growth medium of the cell and/or the amount of CTF99 fragments of APP in cell lysates.

130. The method of claim 129 wherein the cell is from a human, rodent or insect cell line.

131. A method for identifying agents that modulate the activity of human Asp1 aspartyl protease (Hu-Asp1), comprising the steps of:

(a) contacting amyloid precursor protein (APP) and a Hu-Asp1 polypeptide in the presence and absence of a test agent;

(b) determining the APP processing activity of the polypeptide in the presence and absence of the test agent; and

(c) comparing the APP processing activity of the polypeptide in the presence of the test agent to the activity in the absence of the test agent to identify an agent that modulates the APP processing activity of the polypeptide, wherein a modulator that is an Asp1 inhibitor reduces such cleavage and a modulator that is a Asp1 agonist increases such cleavage.

132. A method according to claim 131 wherein the polypeptide is the polypeptide of claim 100.

133. A method according to claim 131, wherein the polypeptide is a recombinant polypeptide purified and isolated from a cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes the polypeptide.

134. A method according to claim 131 or 132, wherein the polypeptide is expressed in a cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes the polypeptide,
wherein the contacting comprises growing the cell in the presence and absence of the test agent, and wherein the determining step comprises measuring APP processing activity of the cell.

135. A method according to claim 134, wherein the determining step comprises measuring the production of amyloid beta peptide by the cell in the presence and absence of the test agent.

136 A method according to claim 134 or 135, wherein the cell is a human embryonic kidney cell line 293 (HEK293) cell.

137. A method according to any one of claims 133-136 wherein the nucleotide sequence is selected from the group consisting of

(a) a nucleotide sequence encoding the Hu-Asp1 amino acid sequence set forth in SEQ ID NO: 1;

(b) a nucleotide sequence encoding a fragment of Hu-Asp1 (SEQ ID NO:1), wherein said fragment exhibits aspartyl protease activity characteristic of Hu-Asp1

(c) a nucleotide sequence of a polynucleotide that hybridizes under stringent hybridization conditions to a Hu-Asp1-encoding polynucleotide having the sequence set forth in SEQ ID NO: 1.

138. A method according to any one of claims 134-137, wherein the cell comprises a vector that comprises the polynucleotide.

139. A method according to any one of claims 131-138, wherein the APP comprises the Swedish mutation (K→ N, M→ L) adjacent to the β-secretase processing site.

140. A method according to any one of claims 131-139, wherein the APP further comprises a carboxy-terminal di-lysine.

141. A method according to any one of claims 131-140, wherein the test agent is an inhibitor

142. A method according to any one of claims 131-140, wherein the test agent is an agonist.

143. A method according to any one of claims 131-142, further comprising a step of treating Alzheimer's Disease with an agent identified as an modulator of Hu-Asp1 according to steps (a)-(c).

144. The use of an agent identified as an inhibitor of Hu-Asp1 according to any one of claims 131-142 in the manufacture of a medicament for the treatment of Alzheimer's Disease.

145. A method of reducing cellular production of amyloid beta (Aβ) from amyloid precursor protein (APP), comprising step of transforming or transfecting cells with an anti-sense reagent capable of reducing Asp1 polypeptide production by reducing Asp1 transcription or translation in the

cells, wherein reduced Asp1 polypeptide production in the cells correlates with reduced cellular processing of APP into Aβ .

146. A method of reducing cellular production of amyloid beta (Aβ) from amyloid precursor protein (APP), comprising steps of:

(a) identifying mammalian cells that produce Aβ ; and

(b) transforming or transfecting the cells with an anti-sense reagent capable of reducing Asp1 polypeptide production by reducing Asp1 transcription or translation in the cells, wherein reduced Asp1 polypeptide production in the cells correlates with reduced cellular processing of APP into Aβ .

147. A method according to claim 146, wherein the identifying step comprises diagnosing Alzheimer's disease, where Alzheimer's disease correlates with the existence of cells that produce Aβ that forms amyloid plaques in the brain.

148. A method according to any one of claims 145-147, wherein the cell is a neural cell.

149. A method according to any one of claims 145-148, wherein the anti-sense reagent comprises an oligonucleotide comprising a single stranded nucleic acid sequence capable of binding to a Hu-Asp1 mRNA.

150. A method for the identification of an agent that decreases the activity of a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), the method comprising

(a) determining the activity of said Hu-Asp polypeptide in the presence of a test agent and in the absence of a test agent; and

(b) comparing the activity of said Hu-Asp polypeptide determined in the presence of said test agent to the activity of said Hu-Asp polypeptide determined in the absence of said test agent;

whereby a lower level of activity in the presence of said test agent than in the absence of said test agent indicates that said test agent has decreased the activity of said Hu-Asp polypeptide..

Statement Under Article 19

The amendment requested is the substitution of application pages 61-78 filed herewith for application pages 61-78 as originally filed. The substitute pages contain new claims 1-150 to replace claims 1-141 as originally filed.

These amendments do not impact the disclosure or drawings in any way. The amended claims all find support throughout the application as originally filed. Thus, the amendments do not go beyond the disclosure of the application as filed.

A non-exhaustive listing of some of the support is pointed out in the letter which accompanies this Statement.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 20 April 2000 (20.04.00)	
International application No. PCT/US99/20881	**Applicant's or agent's file reference** 6177.P CP
International filing date (day/month/year) 23 September 1999 (23.09.99)	**Priority date** (day/month/year) 24 September 1998 (24.09.98)
Applicant GURNEY, Mark, E. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

15 March 2000 (15.03.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Maria Kirchner

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

PERRY, Robert E.
GILL JENNINGS & EVERY
Broadgate House
7 Eldon Street
LONDON EC2M 7LH
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	17.01.2001
Applicant's or agent's file reference: 6177.P CP	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/20881	23/09/1999	24/09/1998

Applicant
PHARMACIA & UPJOHN COMPANY et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Sinanovic, E T l.+31 70 340-2672

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
6177.P CP		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/20881	23/09/1999	24/09/1998
International Patent Classification (IPC) or national classification and IPC C12N15/57		
Applicant PHARMACIA & UPJOHN COMPANY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 18 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15/03/2000	17.01.2001
Name and mailing address of the int mational preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas T l. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Montero Lopez, B Telephon No. +31 70 340 3739

F rm PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/20881

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-60	as originally filed			

Claims, No.:

1-130	as received on	27/11/2000	with letter of	24/11/2000

Drawings, sheets:

1/18-18/18	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

see separate sheet

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 19.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 19.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3, 6, 7, 16, 22, 82-85, 93, 94, 120-123, 126
	No:	Claims	91, 92, 95-105
Inventive step (IS)	Yes:	Claims	82-85, 120-123, 126
	No:	Claims	3, 6, 7, 16, 22, 91-105
Industrial applicability (IA)	Yes:	Claims	3, 6, 7, 16, 22, 82-85, 91-105, 120-123, 126
	No:	Claims	

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I

Basis of the report

1. Pages 1-85 of the sequence listing form part of the description.

2. The amendments filed with the letter dated 24/11/2000 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amended set of claims 1-130 constitute almost in its entirety an entirely different set of claims as it was originally filed, which renders an exhaustive examination thereof for added subject-matter very cumbersome. Nevertheless, multiple features constituting added subject-matter have been found to have been introduced in the claims, of which some examples are here enumerated. The following list of added features is by no means exhaustive:

2.1. "Mammalian" polypeptides: the description refers only to human and murine polypeptides. Other mammalian polypeptides are not disclosed in the application.

2.2. Claim 1 does not include the feature of the Asp2 polypeptide cleaving APP in the β-secretase cleavage site. This feature is presented as essential in the disclosure of the invention. The omission of this feature introduces added subject-matter in claim 1 and other claims which do not include other features to compensate for this omission (claims 11-15, 17, 25-31, 37, 38, and 106-119) and is contrary to Article 19(2)/Article 34(2)(b) PCT.

2.3. Pages 20 and 21 which disclose fragments and variants, refer only to human polypeptides. No reference exists in the application for variants or fragments of murine Asp2, as claimed in claims 2 and 10. Analogously, claim 18 encompasses fragments and variants of the polynucleotides of SEQ ID NOs:5 and 7 not disclosed in the application as filed.

2.4. Claims 4, 5, 8, 9, 20, 21, 23 and 24 refer to polypeptides, and corresponding polynucleotides, lacking aminoacids 1-21 and transmembrane domains which are not specifically disclosed in the application. The mere identification of the sequence corresponding to a signal peptide or a transmembrane domain does not constitute an actual disclosure of polypeptides lacking such sequences.

2.5. Coexpression of APP with a Asp2 polypeptide has been disclosed for very particular circumstances in example 8. Claims 32-36 constitute therefore an undue generalization of example 8 which introduces added subject-matter contrary to Article 34(2)(b) PCT.

2.6. Claims 39-75, 129 and 130 refer to screening methods for modulators of Asp2 aspartyl protease and have been formulated in a way that constitute undue generalizations of the methods disclosed in page 11 and 13, 28 and 29. The claims further include a variety of additional features picked up from various parts of the description and examples, were they have been disclosed in relation with different subject matter than the claimed screening methods. This assembly of various features taking out of their context and put together into a claim constitutes added subject-matter contrary to Article 34(2)(b) PCT.

2.7. Claims 76-81 refer to methods for reducing cellular production of amyloid β-peptide and have been as well formulated in a way that constitute undue generalizations of the method disclosed Example 7.

2.8. Claims 86-90, 124, 125, 127 and 128 refer to a polynucleotide encoding a modified APP according to claims 82-85, 124, 125, 127 and 128. However, the application discloses only three examples thereof, namely, SEQ ID NOs:15, 17, 19. Claims 86-90, 124, 125, 127 and 128 constitute therefore an undue generalization and incorporate added subject-matter contrary to Article 34(2)(b) PCT

Re Item IV

Lack of unity of invention

The present application relates to polypeptides capable of cleaving the beta-secretase cleavage site of APP and polynucleotides encoding them. Polypeptides with aspartic peptidase activity have been characterized in the state of the art (see EP-A-848062). In the light of the prior art a problem underlying the present application can be formulated as providing polypeptides capable of cleaving the beta-secretase cleavage site of APP. The following solution is proposed:

1. Polypeptides Asp-2(a) of SEQ ID NO:4 or 8, Asp-1 of SEQ ID NO:2 and Asp-2(b) of SEQ ID NO:6 and polynucleotides encoding them.

A further problem identified in the application relates to a method for increasing the processing of the amyloid beta peptide from the amyloid protein precursor. The solution proposed as formulated in claims 82-90 and 120-128 is as follows:

2. Providing an APP isoform where the last two carboxy terminus amino acids are Lysine residues.

Given the essential difference between the problems posed and their corresponding solutions, and since in the light of the state of the art, no other technical feature could be distinguished as being new and common to the identified problems and corresponding solutions, the IPEA is of the opinion that there is no single inventive concept underlying the plurality of the claimed inventions in the present application, in the sense of Rule 13.1 PCT. The requisite unity of invention (Rule 13.1 PCT) therefore no longer exists inasmuch as a technical relationship involving one or more of the same or corresponding special technical features in the sense of Rule 13.2 PCT does not exist between the subject-matter of above mentioned groups of claims.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: EP-A-0 848 062 (SMITHKLINE-BEECHAM CORPORATION) 17 June 1998 (1998-06-17) cited in the application

D2: EP-A-0 855 444 (SMITHKLINE-BEECHAM P.L.C.) 29 July 1998 (1998-07-29) cited in the application

1. Claims 3, 6, 7, 16 and 22 refer to a human Asp2(a) sequence of SEQ ID NO:4,

Asp2(b) of sequence SEQ ID NO:6, fragments and variants thereof and polynucleotide encoding SEQ ID NO:4 lacking aminoacids 454-501. Such sequences have not been disclosed in the state of the art and therefore claims 3, 6, 7, 16 and 22 are novel and comply with the requirements of Article 33(2) PCT.

1.1. Document D2 discloses an Aspartic protease designated ASP2 of sequence SEQ ID NO:2 and its encoding nucleic acid of SEQ ID NO:1, sharing respectively 99.8% identity with SEQ ID NO:4 and 94.8% identity with SEQ ID NO:6. Given their high similarity, the claimed Asp2(a) and Asp2(b) polypeptides are considered mere alternative equivalent variants from the sequence disclosed in D2, which do not provide any particular advantage or surprising effect over the sequence provided in the state of the art. Claims 3, 6, and 7 do not therefor involve an inventive step and do not comply with the requirements of Article 33(3) PCT.

1.2. Claim 16 refers to SEQ ID NO:4 or 6 lacking aminoacids 1-45, which corresponds to a fragment after cleavage with a protease. Document D2 discloses the Asp2 polypeptide and variants thereof obtained, for instance, by proteolytic processing (see page 3, par. 2). In particular, table 4 discloses a partial amino acid sequence of human Asp2 lacking amino acids 1-57. Claim 16 is therefore considered not inventive and does not comply with the requirements of Article 33(3) PCT.

1.3. Claim 22 refers to polynucleotide encoding SEQ ID NO:4 lacking aminoacids 454-501, which corresponds to the C-terminal transmembrane domain. Document D2 encompasses fragments of the Asp2 polypeptide lacking the carboxy terminus (page 5, lines 23-26). The determination of the transmembrane domain of a given protein is a standard procedure in the art which the skilled person would put into practice without the need of exercising any inventive step. Consequently, claim 22 does not involve an inventive step and does not comply with the requirements of Article 33(3) PCT.

2. Claims 82-85, 120-123 and 126 refer to isoforms of APP modified to include to Lys residues at the carboxy terminus. No such APP forms have been disclosed in the state of the art and consequently, claims 82-85, 120-123 and 126 are novel and comply with the requirements of Article 33(2) PCT.

2.1. Such a modification has never been suggested in the prior art, and moreover, the presence of two Lys residues at the carboxy terminus of APP involves the unexpected advantage of increasing the processing of the amyloid-β peptide by 2-4 fold. Consequently claims 82-85, 120-123 and 126 involve an inventive step and comply with the requirements of Article 33(3) PCT.

3. Claims 91-99, 104 and 105 are directed to the nucleic acid encoding Hu-Asp1 of SEQ ID NO:2, Hu-Asp2(a) of SEQ ID NO:4 and Hu-Asp2(b) of SEQ ID NO:6, vector and host cells comprising the same.

3.1. Document D1 discloses the nucleotide sequence encoding aspartic protease ASP1 identical to SEQ ID NO:2 of the underlying application (see tables 1 and 2), as well as vectors and host cells comprising the same (page 8, line 45 - page 9, line 26). Claims 91, 92, 95-99, 104 and 105 are therefore not novel and do not comply with the requirements of Article 33(3) PCT.

3.2. Document D2 discloses the nucleotide sequence encoding aspartyl protease Asp2 sharing respectively 99.8% identity with SEQ ID NO:4 and 94.8% identity with SEQ ID NO:6. Given their high similarity, the claimed Asp2(a) and Asp2(b) polypeptides are considered mere alternative equivalent variants from the sequence disclosed in D2, which do not provide any particular advantage or surprising effect over the sequence provided in the state of the art and neither do their encoding nucleic acid sequences. Claims 93 and 94 do not therefor involve an inventive step and do not comply with the requirements of Article 33(3) PCT.

4. Claims 100-102 are directed to polypeptides at least 95% identical to respectively SEQ ID NOs:2, 4 and 8.

4.1. Document D1 discloses ASP1 identical to SEQ ID NO:2 of the underlying application (see table 2). Claim 100 is therefore not novel and does not comply with the requirements of Article 33(2) PCT.

4.2. Document D2 discloses the nucleotide sequence encoding aspartyl protease Asp2 sharing respectively 99.8% identity with SEQ ID NO:4 and 96.2% with SEQ ID NO:8. Claims 101 and 102 are therefore not novel and do not comply with the

requirements of Article 33(2) PCT.

5. Claim 103 refers to antibodies to the polypeptides of claims 100-102. Document D1 discloses antibodies for the Asp1 polypeptide (see page 10, lines 28-44) and therefore claim 103 is not novel and does not comply with the requirements of Article 33(2) PCT.

Re Item VI
Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
PCT/US98/27608	08/07/1999	24/12/1998	31/12/1997
PCT/US99/05028	16/09/1999	08/03/1999	10/03/1997

Re Item VII
Certain defects in the international application

1. The vague and imprecise statement in the description on page 60, lines 18-22 implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, III-4.3a).

Re Item VIII
Certain observations on the international application

1. The large number of the claims presently on file renders it difficult, if not impossible, to determine the matter for which protection is sought and therefore, the present application as a whole fails to comply with the requirements of Article 6 and Rule 6.1(a) PCT. Multiple claims appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which

protection is sought and/or in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection.

2. Claim 1 include a feature defined in terms of a desirable result ("a polypeptide that cleaves a mammalian β-amyloid precursor protein"). Such a definition introduces an unclarity in the scope of the claims, which should state the means for achieving the aimed result. The claims attempts to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem. Claim 1 does not therefore comply with the requirements of Article 6 PCT.

3. It is clear from the description that the amino acid sequence of the Asp2 polypeptides is an essential feature to the definition of the invention. Since independent claim 1 does not contain this feature it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
PHARMACIA & UPJOHN COMPANY
Intellectual Property Legal Serv.
Attn. Wootton, Thomas A.
301 Henrietta Street
Kalamazoo. MI 49001
UNITED STATES OF AMERICA

Date of mailing *(day/month/year)* 02/08/2000	
Applicant's or agent's file reference 6177.P CP	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 99/20881	International filing date *(day/month/year)* 23/09/1999
Applicant PHARMACIA & UPJOHN COMPANY et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Tx. 31 651 epo nl	Andria Overbeeke-Siepkes

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the *international application* may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the *International* Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREA

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
6177.P CP		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/20881	23/09/1999	24/09/1998

Applicant

PHARMACIA & UPJOHN COMPANY et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 8 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/20881

Box I Observations where certain claims were found unsearchable (Continuation f Item 1 f first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 206

Continuation of Box 3.

Claims Nos.: claims 32, 33, 35, 36, 70, 71, 76, 78 and 79 and partially claims 1, 18, 28, 44, 61, 72 and 141

Present claims 1, 18, 28, 44, 61, 72 and 141 relate to an extremely large number of possible products. In fact, the claims encompass so many possible compounds that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible.

Moreover, in view of the large number and also the wording of the claims presently on file, which renders it difficult, if not impossible, to determine the matter for which protection is sought, the present application fails to comply with the clarity and conciseness requirements of Article 6 PCT (see also Rule 6.1(a) PCT) to such an extent that a meaningful search is impossible.

In addition, the obscure definition of claims 32, 33, 35, 36, 70, 71, 76, 78 and 79, relating to an unidentified SEQ ID. and referring to the examples renders as well the search of these claims impracticable.

Consequently, the search has been carried out for those parts of the application which do appear to be clear, namely the particular sequences SEQ ID NOs.: 1, 2, 3, 4, 5, 6, and 8, variants, and uses thereof

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: claims 32, 33, 35, 36, 70, 71, 76, 78 and 79 and partially claims 1, 18, 28, 44, 61, 72 and 141

Present claims 1, 18, 28, 44, 61, 72 and 141 relate to an extremely large number of possible products. In fact, the claims encompass so many possible compounds that a lack of clarity (and/or conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible.

Moreover, in view of the large number and also the wording of the claims presently on file, which renders it difficult, if not impossible, to determine the matter for which protection is sought, the present application fails to comply with the clarity and conciseness requirements of Article 6 PCT (see also Rule 6.1(a) PCT) to such an extent that a meaningful search is impossible.

In addition, the obscure definition of claims 32, 33, 35, 36, 70, 71, 76, 78 and 79, relating to an unidentified SEQ ID. and referring to the examples renders as well the search of these claims impracticable.

Consequently, the search has been carried out for those parts of the application which do appear to be clear, namely the particular sequences SEQ ID NOs.: 1, 2, 3, 4, 5, 6, and 8, variants, and uses thereof

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-31, 34, 37-69, 72-75, 77, 80-129, 136-140 and partially 141

   Proteases capable of cleaving the beta secretase cleavage site of APP, variants thereof; polynucleotides encoding them; vectors and host cells comprising the same; antibodies for the polypeptides and uses of the foregoing in screening tests.

2. Claims: 130-135 and partially 141

   APP isoform wherein the last two carboxy terminus amino acids are Lysine residues.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/20881

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/57 C12N15/62 C12N15/85 C12N5/10 C12N9/64
C07K19/00 C07K14/47 C12N15/12 C07K16/18 C12Q1/37
G01N33/68 C12N1/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, STRAND, WPI Data, BIOSIS, CHEM ABS Data, MEDLINE, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 848 062 A (SMITHKLINE-BEECHAM CORPORATION) 17 June 1998 (1998-06-17) cited in the application page 2, line 10 -page 3, line 40 page 4, line 20 - line 33 page 5, line 8 - line 20 page 8, line 1 -page 9, line 25; tables 1,2 -/--	1-3, 5-21,24, 25, 28-31, 34, 37-47, 49-64, 66-69, 72-75, 77, 80-91, 95-97, 114-129, 140,141

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 July 2000	02.08.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montero Lopez, B

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
	page 10, line 28 - line 44 page 11, line 10 -page 12, line 8 ---	
X	EP 0 855 444 A (SMITHKLINE-BEECHAM P.L.C.) 29 July 1998 (1998-07-29) cited in the application page 2, line 8 -page 3, line 44 page 5, line 3 - line 15 page 5, line 49 -page 6, line 3; tables 1,2 page 7, line 34 - line 50 page 10, line 20 -page 11, line 1 page 12, line 1 - line 19 page 12, line 45 -page 13, line 44 ---	1-3, 5-21,24, 25, 28-31, 34, 37-47, 49-64, 66-69, 72-75, 77, 80-91, 95-97, 114-129, 140,141
X	WO 96 40885 A (ATHENA NEUROSCIENCES) 19 December 1996 (1996-12-19) page 3, line 1 -page 5, line 26 page 8, line 1 - line 34 page 14, line 19 -page 17, line 22 page 23, line 31 -page 25, line 20 page 28, line 7 -page 48, line 13 --- -/--	1-4,6,7, 9,10, 12-21, 24,25, 28-31, 34, 37-47, 49,50, 52,53, 55-63, 67,68, 72-75, 77, 80-90, 108-129, 136-139, 141

3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 26059 A (ATHENA NEUROSCIENCES, INC.) 18 June 1998 (1998-06-18) page 2, line 35 -page 4, line 3 page 5, line 9 -page 11, line 5 page 11, line 10 -page 22, line 3 ---	1-4,6,7, 9,10, 12-21, 24,25, 28-31, 34, 37-47, 49,50, 52,53, 55-63, 67,68, 72-75, 77, 80-90, 108-129, 136-139, 141
P,X	WO 99 34004 A (CHIRON CORPORATION) 8 July 1999 (1999-07-08) page 7, line 19 -page 8, line 9 page 11, line 22 -page 14, line 24 page 16, line 26 -page 21, line 1 page 21, line 20 -page 23, line 13; figure 2; examples 2,3 --- -/--	1-4,6,7, 9-20,24, 28-31, 34, 37-47, 49,50, 52-63, 67,68, 72-75, 77, 80-92, 95-98, 101-103, 106,107, 114-117, 120,141

3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 46281 A (GENENTECH, INC.) 16 September 1999 (1999-09-16) page 15, line 10 - line 23 page 65, line 5 - line 25 page 130, line 30 - line 35 page 149, line 3 -page 155, line 6 page 160, line 20 - line 22 page 173, line 35 -page 175, line 23; figures 72,73; examples 32,99-107	1-4,6,7, 9-12, 18-20, 24, 28-31, 34,37, 38, 40-47, 49,50, 52-54, 61-63, 67,68, 72-75, 77,80, 81, 84-92, 95-98, 101-103, 106,107, 114-118, 120-128, 140,141
A	US 5 795 963 A (MICHAEL JOHN MULLAN) 18 August 1998 (1998-08-18) column 3, line 58 -column 6, line 21	130-135, 141
T	YAN RIQIANG ET AL.: "Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity" NATURE, vol. 402, 2 December 1999 (1999-12-02), pages 533-537, XP002136300 LONDON GB	

3

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/20881

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 848062 A	17-06-1998	JP 11069981 A	16-03-1999
		US 6025180 A	15-02-2000
EP 855444 A	29-07-1998	CA 2221686 A	28-07-1998
		JP 10327875 A	15-12-1998
		JP 2000060579 A	29-02-2000
WO 9640885 A	19-12-1996	US 5744346 A	28-04-1998
		AU 6383396 A	30-12-1996
		EP 0871720 A	21-10-1998
		JP 11507538 T	06-07-1999
		US 5942400 A	24-08-1999
WO 9826059 A	18-06-1998	AU 1684097 A	03-07-1998
WO 9934004 A	08-07-1999	AU 1726199 A	19-07-1999
		AU 2014899 A	19-07-1999
		WO 9933963 A	08-07-1999
WO 9946281 A	16-09-1999	AU 3072199 A	27-09-1999
		AU 3075099 A	11-10-1999
		WO 9947677 A	23-09-1999
		AU 1532499 A	15-06-1999
		WO 9927098 A	03-06-1999
		AU 3757099 A	08-11-1999
		WO 9954467 A	28-10-1999
		AU 1070399 A	10-05-1999
		WO 9920756 A	29-04-1999
US 5795963 A	18-08-1998	US 5455169 A	03-10-1995

What is claimed is:

1. Any isolated or purified nucleic acid polynucleotide that codes for a protease capable of cleaving the beta (β) secretase cleavage site of APP that contains two or more sets of special nucleic acids, where the special nucleic acids are separated by nucleic acids that code for about 100 to 300 amino acid positions, where the amino acids in those positions may be any amino acids, where the first set of special nucleic acids consists of the nucleic acids that code for the peptide DTG, where the first nucleic acid of the first special set of nucleic acids is, the first special nucleic acid, and where the second set of nucleic acids code for either the peptide DSG or DTG, where the last nucleic acid of the second set of nucleic acids is the last special nucleic acid, with the proviso that the nucleic acids disclosed in SEQ ID NO. 1 and SEQ. ID NO. 5 are not included.

2. The nucleic acid polynucleotide of claim 1 where the two sets of nucleic acids are separated by nucleic acids that code for about 125 to 222 amino acid positions, which may be any amino acids.

3. The nucleic acid polynucleotide of claim 2 that code for about 150 to 172 amino acid positions, which may be any amino acids.

4. The nucleic acid polynucleotide of claim that code for about 172 amino acid positions, which may be any amino acids.

5. The nucleic acid polynucleotide of claim 4 where the nucleotides are described in SEQ. ID. NO. 3

6. The nucleic acid polynucleotide of claim 2 where the two sets of nucleic acids are separated by nucleic acids that code for about 150 to 196 amino acid positions.

7. The nucleic acid polynucleotide of claim 6 where the two sets of nucleotides are separated by nucleic acids that code for about 196 amino acids (positions).

8. The nucleic acid polynucleotide of claim 7 where the two sets of nucleic acids are separated by the same nucleic acid sequences that separate the same set of special nucleic acids in SEQ. ID. NO. 5.

9. The nucleic acid polynucleotide of claim 4 where the two sets of nucleic acids are separated by nucleic acids that code for about 150 to 190, amino acid (positions).

10. The nucleic acid polynucleotide of claim 9 where the two sets of nucleotides are separated by nucleic acids that code for about 190 amino acids (positions).

11. The nucleic acid polynucleotide of claim 10 where the two sets of nucleotides are separated by the same nucleic acid sequences that separate the same set of special nucleotides in SEQ. ID. NO. 1.

12. Claims 1-11 where the first nucleic acid of the first special set of amino acids, that is, the first special nucleic acid, is operably linked to any codon where the nuclic acids of that codon codes for any peptide comprising from 1 to 10,000 amino acid (positions).

13. The nucleic acid polynucleotide of claims 1-12 where the first special nucleic acid is operably linked to nucleic acid polymers that code for any peptide selected from the group consisting of: any any reporter proteins or proteins which facilitate purification.

14. The nucleic acid polynucleotide of claims 1-13 where the first special nucleic acid is operably linked to nucleic acid polymers that code for any peptide selected from the group consisting of: immunoglobin-heavy chain, maltose binding protein, glutathion S transfection, Green Fluorescent protein, and ubiquitin.

15. Claims 1-14 where the last nucleic acid of the second set of special amino acids, that is, the last special nucleic acid, is operably linked to nucleic acid polymers that code for any peptide comprising any amino acids from 1 to 10,000 amino acids.

16. Claims 1-15 where the last special nucleic acid is operably linked to any codon linked to nucleic acid polymers that code for any peptide selected from the group consisting of: any reporter proteins or proteins which facilitate purification.

17. The nucleic acid polynucleotide of claims 1-16 where the first special nucleic acid is operably linked to nucleic acid polymers that code for any peptide selected from the group consisting of: immunoglobin-heavy chain, maltose binding protein, glutathion S transfection, Green Fluorescent protein, and ubiquitin.

18. * Any isolated or purified nucleic acid polynucleotide that codes for a protease capable of cleaving the beta secretase cleavage site of APP that contains two or more sets of special nucleic acids, where the special nucleic acids are separated by nucleic acids that code for about 100 to 300 amino acid positions, where the amino acids in those positions may be any amino acids, where the first set of special nucleic acids consists of the nucleic acids that code for DTG, where the first nucleic acid of the first special set of nucleic acids is, the first special nucleic acid, and where the second set of nucleic acids code for either DSG or DTG, where the last nucleic acid of the second set of special nucleic acids is the last special nucleic acid, where the first special nucleic acid is operably linked to nucleic acids that code for any number of amino acids from zero to 81 amino acids and where each of those codons may code for any amino acid.

19. The nucleic acid polynucleotide of claim 18 , where the first special nucleic acid is operably linked to nucleic acids that code for any number of from 64 to 77 amino acids where each codon may code for any amino acid.

20. The nucleic acid polynucleotide of claim 19 , where the first special nucleic acid is operably linked to nucleic acids that code for about 71 amino acids peptide.

21. The nucleic acid polynucleotide of claim 20, where the first special nucleic acid is operably linked to 71 amino acid peptide and where the first of those 71 amino acids is the amino acid T.

22. The nucleic acid polynucleotide of claim 21, where the polynucleotide comprises a sequence that is at least 95% identical to the same corresponding amino acids in SEQ. ID. NO. 3, that is, identical to the sequences in SEQ. ID. NO. 3 including the sequences from both the first and or the second special nucleic acids, toward the N-Terminal, through and including 71 amino acids, see Example 10, beginning from the DTG site and including the nucleotides from that code for 71 amino acids).

23. The nucleic acid polynucleotide of claim 22, where the complete polynucleotide comprises identical to the same corresponding amino acids in SEQ. ID. NO. 3, that is, identical to the sequences in SEQ. ID. NO. 3 including the sequences from both the first and or the second special nucleic acids, toward the N-Terminal, through and including 71 amino acids, see Example 10, beginning from the DTG site and including the nucleotides from that code for 71 amino acids).

24. The nucleic acid polynucleotide of claim 18 , where the first special nucleic acid is operably linked to nucleic acids that code for any number of from about 30 to 54 amino acids where each codon may code for any amino acid.

25. The nucleic acid polynucleotide of claim 20, where the first special nucleic acid is operably linked to 47 codons where the first those 35 or 47 amino acids is the amino acid E or G.

26. The nucleic acid polynucleotide of claim 21, where the polynucleotide comprises a sequence that is at least 95% identical to the same corresponding amino acids in SEQ. ID. NO. 3, that is, identical to that portion of the sequences in SEQ. ID. NO. 3 including the sequences from both the first and or the second special nucleic acids, toward the N-Terminal, through and including 35 or 47 amino acids, see Example 11 for the 47 example, beginning from the DTG site and including the nucleotides from that code for the previous 35 or 47 amino acids before the DTG site).

27. The nucleic acid polynucleotide of claim 22, where the polynucleotide comprises identical to the same corresponding amino acids in SEQ. ID. NO. 3, that is, identical to the sequences in SEQ. ID. NO. 3 including the sequences from both the first and or the second special nucleic acids, toward the N-Terminal, through and including 35 or 47 amino acids, see Example 11 for the 47 example, beginning from the DTG site and including the nucleotides from that code for the previous 35 or 47 amino acids before the DTG site).

28. * Any isolated or purified nucleic acid polynucleotide that codes for a protease capable of cleaving the beta (β) secretase cleavage site of APP that contains two or more sets of special nucleic acids, where the special nucleic acids are separated by nucleic acids that code for about 100 to 300 amino acid positions, where the amino acids in those positions may be any amino acids, where the first set of special nucleic acids consists of the nucleic acids that code for the peptide DTG, where the first nucleic acid of the first special set of amino acids is, the first special nucleic acid, and where the second set of special nucleic acids code for either the peptide DSG or DTG, where the last nucleic acid of the second set of special nucleic acids, the last special nucleic acid, is operably linked to nucleic acids that code for any number of codons from 50 to 170 codons.

29. The nucleic acid polynucleotide of claim 29 where the last special nucleic acid is operably linked to nucleic acids comprising from 100 to 170 codons.

30. The nucleic acid polynucleotide of claim 30 where the last special nucleic acid is operably linked to nucleic acids comprising from 142 to 163 codons.

31. The nucleic acid polynucleotide of claim 31 where the last special nucleic acid is operably linked to nucleic acids comprising about 142 codons.

32. The nucleic acid polynucleotide of claim 32 where the polynucleotide comprises a sequence that is at least 95% identical to SEQ. ID. # (Example 9 or 10).

33. The nucleic acid polynucleotide of claim 33, where the complete polynucleotide comprises SEQ. ID. # (Example 9 or 10).

34. The nucleic acid polynucleotide of claim 31 where the last special nucleic acid is operably linked to nucleic acids comprising about 163 codons.

35. The nucleic acid polynucleotide of claim 35 where the polynucleotide comprises a sequence that is at least 95% identical to SEQ. ID. # (Example 9 or 10).

36. The nucleic acid polynucleotide of claim 36, where the complete polynucleotide comprises SEQ. ID. # (Example 9 or 10).

37. The nucleic acid polynucleotide of claim 31 where the last special nucleic acid is operably linked to nucleic acids comprising about 170 codons.

38. Claims 1-38 where the second set of special nucleid acids code for the peptide DSG, and optionally the first set of nucleic acid polynucleotide is operably linked to a peptide purification tag.

39. Claims 1-39 where the nucleic acid polynucleotide is operably linked to a peptide purification tag which is six histidine.

40. Claims 1-40 where the first set of special nucleic acids are on one polynucleotide and the second set of special nucleic acids are on a second polynucleotide, where both first and second polynucleotides have at lease 50 codons.

41. Claims 1-40 where the first set of special nucleic acids are on one polynucleotide and the second set of special nucleic acids are on a second polynucleotide, where both first and second polynucleotides have at lease 50 codons where both said polynucleotides are in the same solution.

42. A vector which contains a polynucleotide described in claims 1-42.

43. A cell or cell line which contans a polynucleotide described in claims 1-42.

44. Any isolated or purified peptide or protein comprising an amino acid polymer that is a protease capable of cleaving the beta (β) secretase cleavage site of APP that contains two or more sets of special amino acids, where the special amino acids are separated by about 100 to 300 amino acid positions, where each amino acid position can be any amino acid, where the first set of special amino acids consists of the peptide DTG, where the first amino acid of the first special set of amino acids is, the first special amino acid, where the second set of amino acids is selected from the peptide comprising either DSG or DTG, where the last amino acid of the second set of special amino acids is the last special amino acid, with the proviso that the proteases disclosed in SEQ ID NO. 2 and SEQ. ID NO. 6 are not included.

45. The amino acid polypeptide of claim 45 where the two sets of amino acids are separated by about 125 to 222 amino acid positions where in each position it may be any amino acid.

46. The amino acid polypeptide of claim 46 where the two sets of amino acids are separated by about 150 to 172 amino acids.

47. The amino acid polypeptide of claim 47 where the two sets of amino acids are separated by about 172 amino acids.

48. The amino acid polypeptide of claim 48 where the protease is described in SEQ. ID. NO. 4

49. The amino acid polypeptide of claim 46 where the two sets of amino acids are separated by about 150 to 196 amino acids.

50. The amino acid polypeptide of claim 50 where the two sets of amino acids are separated by about 196 amino acids.

51. The amino acid polypeptide of claim 51 where the two sets of amino acids are separated by the same amino acid sequences that separate the same set of special amino acids in SEQ. ID. NO. 6.

52. The amino acid polypeptide of claim 46 where the two sets of amino acids are separated by about 150 to 190, amino acids.

53. The amino acid polypeptide of claim 53 where the two sets of nucleotides are separated by about 190 amino acids.

54. The amino acid polypeptide of claim 54 where the two sets of nucleotides are separated by the same amino acid sequences that separate the same set of special amino acids in SEQ. ID. NO. 2.

55. Claims 45-55 where the first amino acid of the first special set of amino acids, that is, the first special amino acid, is operably linked to any peptide comprising from 1 to 10,000 amino acids.

56. The amino acid polypeptide of claims 45-56 where the first special amino acid is operably linked to any peptide selected from the group consisting of: any any reporter proteins or proteins which facilitate purification.

57. The amino acid polypeptide of claims 45-57 where the first special amino acid is operably linked to any peptide selected from the group consisting of: immunoglobin-heavy chain, maltose binding protein, glutathion S transfection, Green Fluorescent protein, and ubiquitin.

58. Claims 45-58, where the last amino acid of the second set of special amino acids, that is, the last special amino acid, is operably linked to any peptide comprising any amino acids from 1 to 10,000 amino acids.

59. Claims 45-59 where the last special amino acid is operably linked any peptide selected from the group consisting of any reporter proteins or proteins which facilitate purification.

60. The amino acid polypeptide of claims 45-60 where the first special amino acid is operably linked to any peptide selected from the group consisting of: immunoglobin-heavy chain, maltose binding protein, glutathion S transfection, Green Fluorescent protein, and ubiquitin.

61. • Any isolated or purified peptide or protein comprising an amino acid polypeptide that codes for a protease capable of cleaving the beta secretase cleavage site of APP that contains two or more sets of special amino acids, where the special amino acids are separated by about 100 to 300 amino acid positions, where each amino acid in each position can be any amino acid, where the first set of special amino acids consists of the amino acids DTG, where the first amino acid of the first special set of amino acids is, the first special amino acid, D, and where the second set of amino acids is either DSG or DTG, where the last amino acid of the second set of special amino acids is the last special amino acid, G, where the first special amino acid is operably linked to amino acids that code for any number of amino acids from zero to 81 amino acid positions where in each position it may be any amino acid.

62. The amino acid polypeptide of claim 62, where the first special amino acid is operably linked to a peptide from about 30 to 77 amino acids positions where each amino acid position may be any amino acid.

63. The amino acid polypeptide of claim 63, where the first special amino acid is operably linked to a peptide of 35, 47, 71, or 77 amino acids.

64. The amino acid polypeptide of claim 63, where the first special amino acid is operably linked to the same corresponding peptides from SEQ. ID. NO. 3 that are 35, 47, 71, or 77 peptides in length, beginning counting with the amino acids on the first special sequence, DTG, towards the N-terminal of SEQ. ID. NO. 3.

65. The amino acid polypeptide of claim 65, where the polypeptide comprises a sequence that is at least 95% identical to the same corresponding amino acids in SEQ. ID. NO. 4, that is, identical to that portion of the sequences in SEQ.ID. NO. 4, including all the sequences from both the first and or the second special nucleic acids, toward the N- terminal, through and including 71, 47, 35 amino acids before the first special amino acids. (Examples 10 and 11).

66. The amino acid polypeptide of claim 65, where the complete polypeptide comprises the peptide of 71 amino acids, where the first of the amino acid is T and the second is Q.

67. The amino acid polypeptide of claim 62, where the first special amino acid is operably linked to any number of from 40 to 54 amino acids (positions) where each amino acid position may be any amino acid.

68. The amino acid polypeptide of claim 68, where the first special amino acid is operably linked to amino acids that code for a peptide of 47 amino acids.

69. The amino acid polypeptide of claim 69, where the first special amino acid is operably linked to a 47 amino acid peptide where the first those 47 amino acids is the amino acid E.

70. The amino acid polypeptide of claim 70, where the polypeptide comprises a sequence that is at least 95% identical to SEQ. ID. # (Example 10).

71. The amino acid polypeptide of claim 71, where the complete polypeptide comprises SEQ. ID. # (Example 10).

72. * Any isolated or purified amino acid polypeptide that is a protease capable of cleaving the beta (β) secretase cleavage site of APP that contains two or more sets of special amino acids, where the special amino acids are separated by about 100 to 300 amino acid positions, where each amino acid in each position can be any amino

acid, where the first set of special amino acids consists of the amino acids that code for DTG, where the first amino acid of the first special set of amino acids is, the first special amino acid, D, and where the second set of amino acids are either DSG or DTG, where the last amino acid of the second set of special amino acids is the last special amino acid, G, which is operably linked to any number of amino acids from 50 to 170 amino acids, which may be any amino acids.

73. The amino acid polypeptide of claim 73 where the last special amino acid is operably linked to a peptide of about 100 to 170 amino acids.

74. The amino acid polypeptide of claim 74 where the last special amino acid is operably linked to to a peptide of about 142 to 163 amino acids.

75. The amino acid polypeptide of claim 75 where the last special amino acid is operably linked to to a peptide of about about 142 amino acids.

76. The amino acid polypeptide of claim 76 where the polypeptide comprises a sequence that is at least 95% identical to SEQ. ID. # (Example 9 or 10).

77. The amino acid polypeptide of claim 75 where the last special amino acid is operably linked to a peptide of about 163 amino acids.

78. The amino acid polypeptide of claim 79 where the polypeptide comprises a sequence that is at least 95% identical to SEQ. ID. # (Example 9 or 10).

79. The amino acid polypeptide of claim 79, where the complete polypeptide comprises SEQ. ID. # (Example 9 or 10).

80. The amino acid polypeptide of claim 74 where the last special amino acid is operably linked to to a peptide of about 170 amino acids.

81. Claim 46-81 where the second set of special amino acids is comprised of the peptide with the amino acid sequence DSG.

82. Claims 45-82 where the amino acid polypeptide is operably linked to a peptide purification tag.

83. Claims 45-83 where the amino acid polypeptide is operably linked to a peptide purification tag which is six histidine.

84. Claims 45-84 where the first set of special amino acids are on one polypeptide and the second set of special amino acids are on a second polypeptide, where both first and second polypeptide have at lease 50 amino acids, which may be any amino acids.

85. Claims 45-84 where the first set of special amino acids are on one polypeptide and the second set of special amino acids are on a second polypeptide, where both first and second polypeptides have at lease 50 amino acids where both said polypeptides are in the same vessel.

86. A vector which contains a polypeptide described in claims 45-86.

87. A cell or cell line which contans a polynucleotide described in claims 45-87.

88. The process of making any of the polynucleotides, vectors, or cells of claims 1-44

89. The process of making any of the polypeptides, vectors or cells of claims 45-88

90. Any of the polynucleotides, polypeptides, vectors, cells or cell lines described in claims 1-88 made from the processes described in claims 89 and 90.

91. * An isolated nucleic acid molecule comprising a polynucleotide, said polynucleotide encoding a Hu-Asp polypeptide and having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), wherein said Hu-Asp1, Hu-Asp2(a) and Hu-Asp2(b) polypeptides have the complete amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID No:6, respectively; and

(b) a nucleotide sequence complementary to the nucleotide sequence of (a).

92. The nucleic acid molecule of claim 92, wherein said Hu-Asp polypeptide is Hu-Asp1, and said polynucleotide molecule of 1(a) comprises the nucleotide sequence of SEQ ID NO:1.

93. The nucleic acid molecule of claim 92, wherein said Hu-Asp polypeptide is Hu-Asp2(a), and said polynucleotide molecule of 1(a) comprises the nucleotide sequence of SEQ ID NO:4.

94. The nucleic acid molecule of claim 92, wherein said Hu-Asp polypeptide is Hu-Asp2(b), and said polynucleotide molecule of 1(a) comprises the nucleotide sequence of SEQ ID NO:5.

95. An isolated nucleic acid molecule comprising polynucleotide which hybridizes under stringent conditions to a polynucleotide having the nucleotide sequence in (a) or (b) of claim 92.

96. A vector comprising the nucleic acid molecule of claim 96.

97. The vector of claim 97, wherein said nucleic acid molecule is operably linked to a promoter for the expression of a Hu-Asp polypeptide.

98. The vector of claim 97, wherein said Hu-Asp polypeptide is Hu-Asp1.

99. The vector of claim 97, wherein said Hu-Asp polypeptide is Hu-Asp2(a).

100. The vector of claim 97, wherein said Hu-Asp polypeptide is Hu-Asp2(b).

101. A host cell comprising the vector of claim 98.

102. A method of obtaining a Hu-Asp polypeptide comprising culturing the host cell of claim 102 and isolating said Hu-Asp polypeptide.

103. An isolated Hu-Asp1 polypeptide comprising an amino acid sequence at least 95% identical to a sequence comprising the amino acid sequence of SEQ ID NO:2.

104. An isolated Hu-Asp2(a) polypeptide comprising an amino acid sequence at least 95% identical to a sequence comprising the amino acid sequence of SEQ ID NO:4.

105. An isolated Hu-Asp2(a) polypeptide comprising an amino acid sequence at least 95% identical to a sequence comprising the amino acid sequence of SEQ ID NO:8.

106 An isolated antibody that binds specifically to the Hu-Asp polypeptide of any of claims 104-107.
sequence comprising the amino acid sequence of SEQ ID NO:8.

107 An isolated antibody that binds specifically to the Hu-Asp polypeptide of any of claims 104-107.

108. * A method to identify a cell that can be used to screen for inhibitors of β secretase activity comprising:

a) identifying a cell that expresses a protease capable of cleaving APP at the β secretase site,
comprising:

i) collect the cells or the supernantent from the cells to be identified

ii) measure the production of a critical peptide, where the critical peptide is selected from the group consisting of either the APP C-terminal peptide or soluble APP.

iii) select the cells which produce the critical peptide.

109. The method of claim 108 where the cells are collected and the critical peptide is the APP C-terminal peptide created as a result of the β secretase cleavage.

110. The method of claim 108 where the supernantent is collected and the critical peptide is soluble APP where the soluble APP has a C-terminal created by β secretase cleavage.

111. The method of claim 108 where the cells contain any of the nucleic acids or polypeptides of claims 1-86 and where the cells are shown to cleave the β secretase site of any peptide having the following peptide structure, P2, P1, P1', P2', where P2 is K or N, where P1 is M or L, where P1' is D, where P2' is A.

112. The method of claim 111 where P2 is K and P1 is M.

113 The method of claim 112 where P2 is N and P1 is L.

114 * Any bacterial cell comprising any nucleic acids or peptides in claims 1-86 and 92-107.

115 A bacterial cell of claim 114 where the bacteria is *E coli*.

116 Any eukaryotic cell comprising any nucleic acids or polypeptides in claims 1-86 and 92-107.

117 * Any insect cell comprising any of the nucleic acids or polypeptides in claims 1-86 and 92-107.

118 A insect cell of claim 117 where the insect is sf9, or High 5.

119 A insect cell of claim 100 where the insect cell is High 5.

120 A mammalian cell comprising any of the nucleic acids or polypeptides in claims 1-86 and 92-107.

121 A mammalian cell of claim 120 where the mammalian cell is selected from the group consisting of, human, rodent, lagomorph, and primate.

122 A mammalian cell of claim 121 where the mammalian cell is selected from the group consisting of human cell.

123 A mammalian cell of claim 122 where the human cell is selected from the group comprising HEK293, and IMR-32.

124 A mammalian cell of claim 121 where the cell is a primate cell.

125 A primate cell of claim 124 where the primate cell is a COS-7 cell.

126 A mammalian cell of claim 121 where cell is selected from a rodent cells.

127 A rodent cell of claim 126 selected from, CHO-K1, Neuro-2A, 3T3 cells.

128 A yeast cell of claim 115.

129 An avian cell of claim 115.

130. * Any isoform of APP where the last two carboxy terminus amino acids of that isoform are both lysine residues.

131 The isoform of APP from claim 130 comprising the isoform known as APP695 modified so that its last two having two lysine residues as its last two carboxy terminus amino acids.

132 The isoform of claim 131 comprising SEQ. ID. 16.

133 The isoform variant of claim 1301comprising SEQ. ID. NO. 18, and 20.

134 Any eukaryotic cell line, comprising nucleic acids or polypeptides of claim 130-133.

135 Any cell line of claim 134 that is a mammaliam cell line (HEK293, Neuro2a, are preferred plus any others.)

136 A method for identifying inhibitors of an enzyme that cleaves the beta secretase cleavabe site of APP comprising:

a) culturing cells in a culture medium under conditions in which the enzyme causes processing of APP and release of amyloid beta-peptide into the medium and causes the accumulation of CTF99 fragments of APP in cell lysates,

b) exposing the cultured cells to a test compound; and specifically determining whether the test compound inhibits the function of the enzyme by measuring the amount of amyloid beta-peptide released into the medium and or the amount of CTF99 fragments of APP in cell lysates;

c) identifying test compounds diminishing the amount of soluble amyloid beta peptide present in the culture medium and diminution of CTF99 fragments of APP in cell lysates as Asp2 inhibitors.

137 The method of claim 136 wherein the cultured cells are a human, rodent or insect cell line.

138 The method of claim 137 wherein the human or rodent cell line exhibits β secretase activity in which processing of APP occurs with release of amyloid beta-peptide into the culture medium and accumulation of CTF99 in cell lysates.

139. A method as in claim 138 wherein the human or rodent cell line treated with the antisense oligomers directed against the enzyme that exhibits β secretase activity, reduces release of soluble amyloid beta-peptide into the culture medium and accumulation of CTF99 in cell lysates.

140. A method for the identification of an agent that decreases the activity of a Hu-Asp polypeptide selected from the group consisting of Hu-Asp1, Hu-Asp2(a), and Hu-Asp2(b), the method comprising

(a) determining the activity of said Hu-Asp polypeptide in the presence of a test agent and in the absence of a test agent; and

(b) comparing the activity of said Hu-Asp polypeptide determined in the presence of said test agent to the activity of said Hu-Asp polypeptide determined in the absence of said test agent;

whereby a lower level of activity in the presence of said test agent than in the absence of said test agent indicates that said test agent has decreased the activity of said Hu-Asp polypeptide..

141. The nucleic acids, peptides, proteins, vectors, cells and cell lines, and assays described herein.